

APPENDIX
VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE CLAIMS:

Claims 31, 35 and 49 are canceled.

The claims 30, 32-34, 36-48 and 50 are amended as follows:

30. A method for assaying for a specific nucleic acid sequence that is within a target RNA, wherein said target RNA is a single-stranded RNA [in a sample, wherein said RNA contains a specific nucleic acid sequence], said method comprising the following steps:

- (1) providing a single-stranded RNA comprising said specific nucleic acid sequence;
- (2) [exposing] hybridizing said [first sequence] target RNA to a reagent (A), which is a single-stranded oligo nucleic acid complementary to a sequence 5' of, and adjacent to, the 5' end of said specific nucleic acid sequence that is within the target RNA, which allows the [single-stranded]target RNA to be cut at the 5' end of the specific nucleic acid sequence by the action of a reagent (D), which is a ribonuclease that degrades RNA in a DNA-RNA double-strand;
- (3) cutting the [single-stranded] target RNA at the 5' end of the specific nucleic acid sequence to give a product;
- (4) hybridizing to said product of step (3), a reagent (B), which is a first single-stranded oligo DNA primer complementary to a sequence at the 3' end of said specific nucleic acid sequence;

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- (5) extending said first single-stranded oligo DNA primer to the 5' end of the specific nucleic acid sequence with a reagent (C), which is an RNA-dependent DNA polymerase and with a reagent (E), which is deoxynucleoside triphosphates, to form a DNA-RNA double-strand;
- (6) digesting the RNA strand of said DNA-RNA double-strand from step (5) with the [a] reagent (D), [which is a ribonuclease that degrades RNA in a DNA-RNA double-strand] to give a single-stranded DNA complementary to said specific nucleic acid sequence;
- (7) hybridizing to said single-stranded DNA from step (6) a reagent which is a second single-stranded oligo DNA primer having the following sequences, in the following order, beginning at the 5' end and proceeding in a 5' to 3' direction: i) a promoter sequence for a DNA-dependent RNA polymerase, ii) an enhancer sequence for said promoter sequence, and iii) a sequence at the 5' end of said specific nucleic acid sequence;
- (8) extending said second oligo DNA primer to the 5' end of said single-stranded DNA with a reagent (G), which is a DNA-dependent DNA polymerase and with said reagent (E);
- (9) synthesizing a single-stranded RNA from said promoter sequence with a reagent (H), which is a DNA-dependent RNA polymerase and a reagent (I), which is ribonucleoside triphosphates;

(10) either:

(c) cycling said single-stranded RNA from step (9) to step (4), or

(d) hybridizing to said single-stranded RNA from step (9) a reagent (J),
which is a single-stranded oligo DNA complementary to said
specific nucleic acid sequence, labeled so that it gives off a
measurable fluorescent signal upon hybridization with a
nucleic acid containing said specific nucleic acid sequence; and

(11) after addition of reagents (A) to (J), measuring at least once a fluorescent signal
from said hybrid formed in step (10) (b);

wherein said reagents (A) to (J) are added to a reaction vessel one by one, in functional
combinations, or all at once.

31. ~~The method according to Claim 30, wherein the reagent (A) is a single-stranded
oligo nucleic acid complementary to a sequence 5' of, and adjacent to, the 5' end of said specific
nucleic acid sequence.~~

32. The method according to Claim ~~31~~30, wherein the reagent (A) is a DNA, and the
method further comprises adding an RNaseH and deactivating the RNaseH by heating or by
addition of an inhibitor prior to addition of the reagent (B).

33. The method according to Claim 32, wherein addition of the reagent (A) is
followed by simultaneous addition of the reagents (B) to (I), and then by addition of the reagent
(J).

34. The method according to Claim 32, wherein addition of the reagent (A) is followed by simultaneous addition of the reagents (B) to (J).

~~35. The method according to Claim 30, wherein the oligo nucleic acid as the reagent (A) is a ribozyme or a DNA enzyme.~~

36. The method according to Claim 30, wherein the reagent (C), an RNA-dependent DNA polymerase, is also the reagent (D), a ribonuclease that degrades RNA in a DNA-RNA double strand.

37. The method according to Claim 30, wherein an enzyme having both an RNA-dependent DNA polymerase activity and a DNA-dependent DNA polymerase activity is used as both the reagents (C) and (G).

38. The method according to Claim 37, wherein the enzyme is avian myoblastoma virus polymerase.

39. The method according to Claim 30, wherein the first and second oligo DNA primers as the reagents (B) and (F) are used at concentrations of from 0.02 to 1 μ M.

40. The method according to Claim 30, wherein the DNA-dependent RNA polymerase as the reagent (H) is at least one enzyme selected from the group consisting of phage SP6 polymerase, phage T3 polymerase, and phage T7 polymerase.

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41. The method according to Claim 30, wherein the single-stranded oligo DNA as the reagent (J) is a DNA which is linked to a fluorescent intercalative dye so that the fluorescent intercalative dye changes its fluorescence characteristic upon hybridization of the DNA with another nucleic acid by intercalating into the resulting double strand.

42. The method according to Claim 30, wherein the single-stranded oligo DNA as the reagent (J) is a DNA which has a 3' end sequence that is not complementary to the specific nucleic acid sequence or has a modified 3' end, and hybridizes to the nucleic acid of Claim 30 having said specific nucleic acid sequence.

43. The method according to Claim 41, wherein the single-stranded oligo DNA as the reagent (J) is a DNA which has a 3' end sequence that is not complementary to the specific nucleic acid sequence or has a modified 3' end, and hybridizes to the nucleic acid of Claim 30 having said specific nucleic acid sequence.

44. The method according to Claim 30, which further comprises a step of detecting or quantifying the single-stranded RNA in the sample based on the measured fluorescent signal or change in the measured fluorescent signal.

45. The method according to Claim 30, wherein all the reagents are chloride-free.

46. The method according to Claim 30, wherein prior to said step (10)(b) acetate is added as a reagent.

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47. The method according to Claim 46, wherein the acetate is magnesium acetate at a concentration of from 5 to 20 mM or potassium acetate at a concentration of from 50 to 200 mM.

48. The method according to Claim 30, wherein prior to said step (10)(b) sorbitol is added as a reagent.

~~49. The method according to claim 30, wherein the oligo nucleic acid as the reagent (A) is DNA.~~

50. The method according to Claim 30, wherein the temperature is selected from the range of from 35 to 60°C.